Discovery and Optimization of Inhibitors of STAT3 Activation for the Treatment of Squamous Cell Carcinoma of the Head and Neck

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Wipf Group Research Topic Seminar

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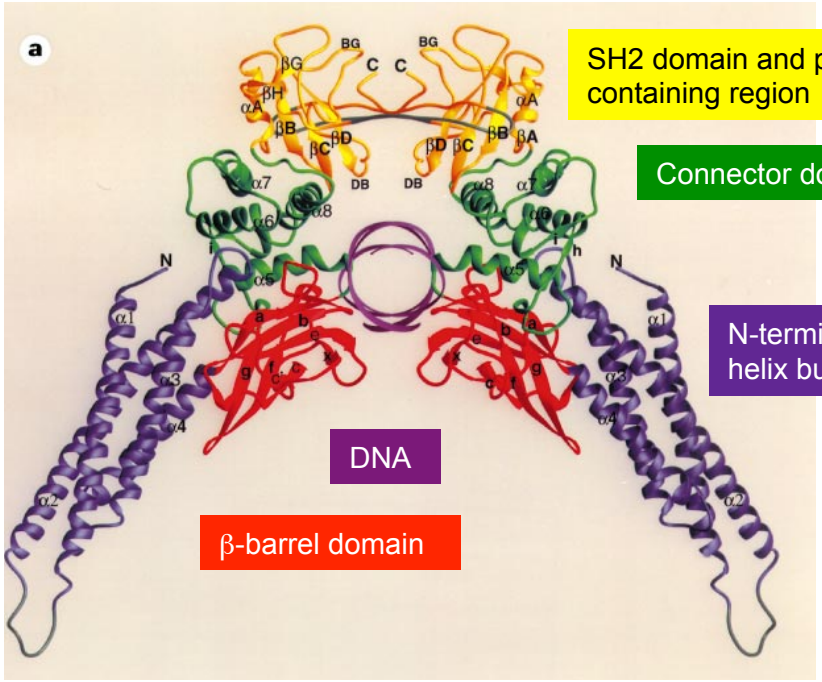
Squamous Cell Carcinoma of the Head and Neck (SCCHN)

- **Squamous Cell Carcinoma of the Head and Neck (SCCHN) is the sixth most common form of cancer and accounts for ~500,000 new cancer cases per year worldwide.**
- **Traditional therapies, including surgery, radiation therapy, and chemotherapy are able to eradicate head and neck cancer in only 50% of cases.**
- **Treatments incorporating radiation or conventional chemotherapy drugs, such as cisplatin, may result in a host of negative side effects, some permanent.**
- **As a result, there has been continuing investigation into potential alternative and less toxic therapies for head and neck cancer, with the aim of achieving a more favorable clinical outcome while reducing treatment morbidity.**
- **Patient-derived primary SCCHN cells and SCCHN cell lines have been shown to overexpress a number of key signaling proteins that contribute to the enhanced growth and survival properties of these cells.**
- **SCCHN cells commonly exhibit overexpression and/or hyperactivation of EGFR, signal transducer and activator of transcription 3 (STAT3), STAT3 is a key downstream target of EGFR**

Signal Transducers and Activators of Transcription

Crystal structure of STAT3 dimer bound to DNA



Becker et al., Nature 1998, 394, 145-151

- ***Conserved tyrosine residue- Y701, Y705 or Y695***

- ***Seven members:***
  - ***STAT1 (α/β), STAT2, STAT3 (α/β/γ), STAT4, STAT5a, STAT5b & STAT6***
  - ***Cytoplasmic transcription factors regulating cytokine gene expression***
  - ***STATs are activated via the tyrosine phosphorylation cascade after ligand binding and stimulation of the Cytokine Receptor–Kinase complex and Growth Factor-Receptor complex***
    - ***EGF (Epidermal Growth Factor), FGF (Fibroblast Growth Factor), PDGF (Platelet-Derived Growth Factor), IL-6 (Interleukin-6), OSM (Oncostatin-M), CSF1R (Colony Stimulating Factor-1 Receptor), c-kit, Insulin receptor, c-Met and GPCRs (G-Protein Coupled Receptors), etc.***

What is STAT3

- **STAT3 is one of seven members of the signal transducer and activator of transcription (STAT) family of proteins whose function is to relay signals from the cell surface receptors to the nucleus and initiate transcription.**

- **STAT3 plays a vital role in regulating cell growth and survival. In response to growth factor and cytokine stimulation, STAT3 is phosphorylated, dimerizes, and translocates into the nucleus to up-regulate transcription of a wide spectrum of genes.**

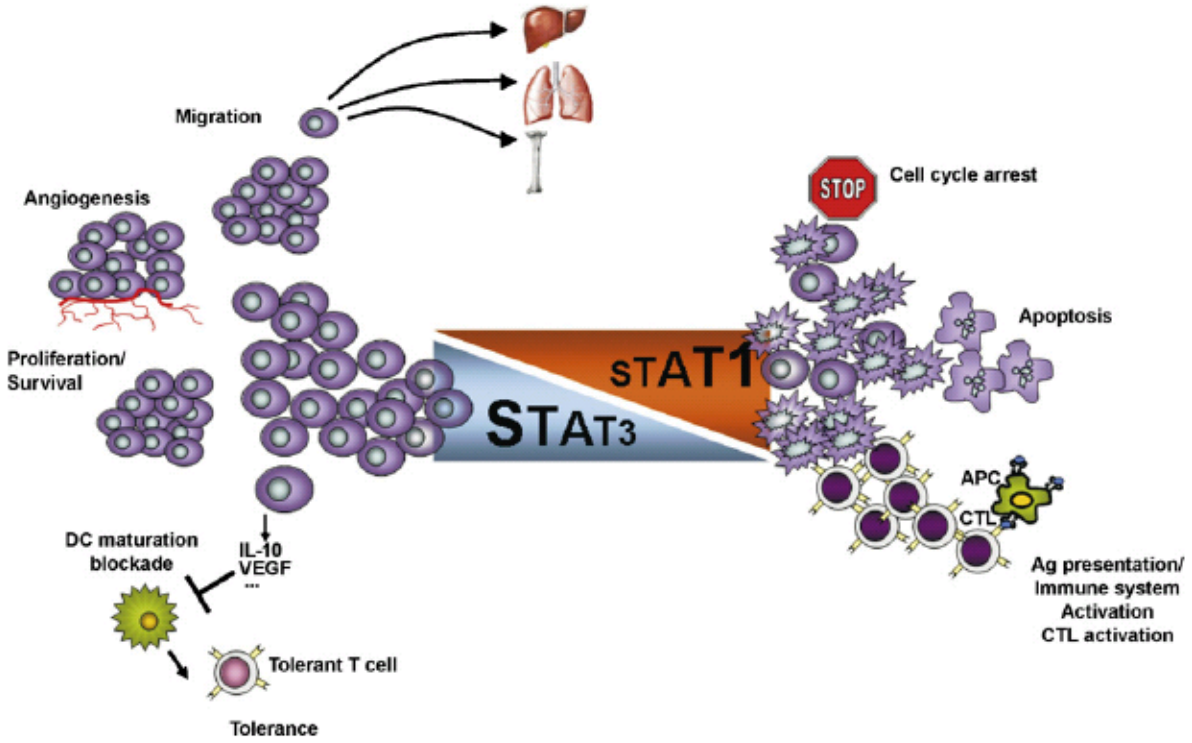
- **STAT3 is a key tumor promoting transcription factor, constitutively activated in many types of cancer including SCCHN, prostate, breast and colorectal cancers.**

- **Activated STAT3 promotes tumor cell proliferation/survival and tumor metastasis, while suppressing the anti-tumor immune response. Inhibition of STAT3 signaling has been shown to inhibit tumor growth in vitro and in vivo.**

The Opposing Roles of STAT3 & STAT1 in Cancer

- **STAT1 has significant sequence and functional similarity to STAT3, however, whereas STAT3 is oncogenic, STAT1 is associated with tumor suppression.**
- **STAT1 can be activated by various ligands including interferon-alpha, interferon-gamma, EGF, PDGF and IL-6.**
- **Activated STAT1 plays a critical role in promoting an effective anti-tumor immune response.**
- **STAT3 inhibitors identified through target-based screening strategies (e.g. SH2-targeting molecules) often exhibit poor cell permeability, efficacy, selectivity (vs STAT1) and/or stability, and have not progressed into the clinic.**
- **STAT3 pathway-specificity (i.e. without STAT1 signaling inhibition) is highly critical when developing anticancer agents designed to block STAT3 activation.**
- **The ultimate goal is to discover and develop selective inhibitors of the STAT3 pathway for the treatment of SCCHN tumors.**

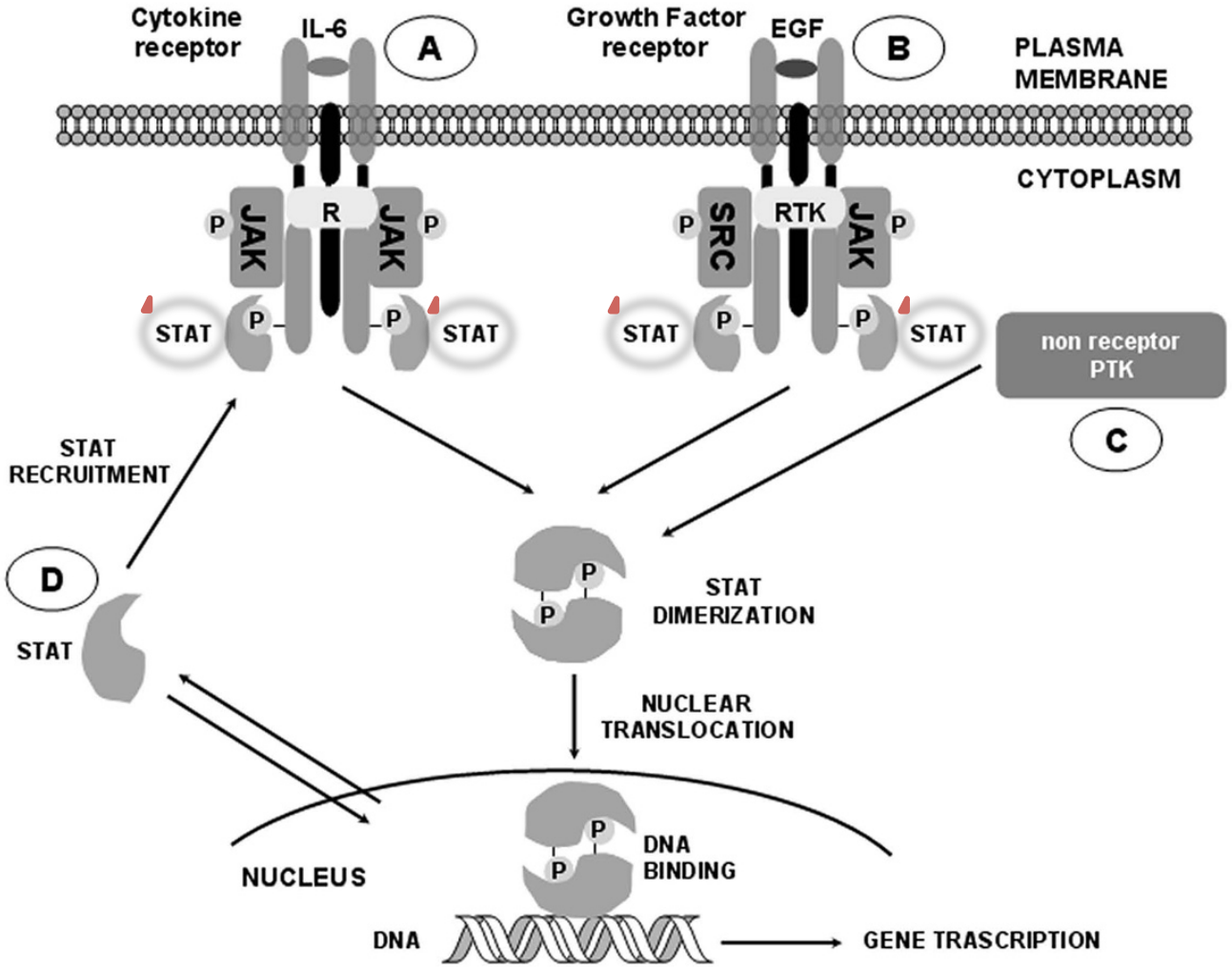
The Opposing Roles of STAT3 & STAT1 in Cancer



- ***STAT3 is a proto-oncogene***
- ***STAT1 is a tumor suppressor***

G. Regis et al. Semin. Cell Dev. Biol. ***2008****, 19, 351–359.*

STAT3 Pathway



Lavecchia, A. et al. Curr. Med. Chem. ***2011****, 18, 2359-2375.*

STAT3 Signaling Pathway as a Therapeutic Target in Cancer

Table 1. STAT3 in the Context of Various Cancers: Validation as an Anticancer Target

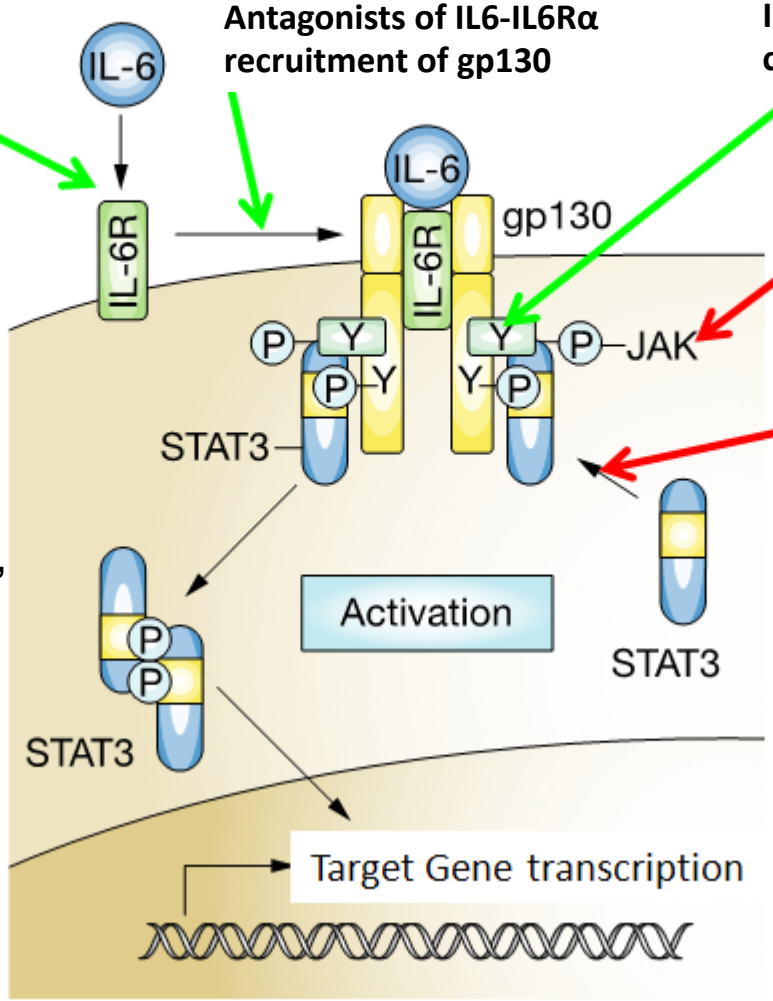
Cancers Characterized by Elevated STAT3 Expression or Activity	Poor Prognosis Linked to High STAT3 Levels	Upstream/Downstream Abnormalities of STAT3 Signaling	Xenograft Models Responsive to Inhibition of STAT3
Leukemia	Renal cell carcinoma	Elevated EGFR expression	Head and neck squamous cell carcinoma
Lymphomas	Colorectal cancer	Constitutively activated EGFR-RTK	Gliobastoma
Multiple myeloma	Ovarian carcinoma	Overexpression of SFKs	Myeloproliferative neoplasms
Breast cancer	Gastric carcinoma	Hyperactivated JAKs	Renal cell carcinoma
Prostate carcinoma	Intestinal-type gastric adenocarcinoma	Elevated TGFα/IL-6	Breast cancer
Lung cancer (non-small-cell)	Cervical squamous-cell carcinoma		Lung adenocarcinoma
Renal cell carcinoma lung cancer	Osteosarcoma		Acute lymphoblastic leukemia
Hepatocellular carcinoma	Epithelial ovarian carcinoma		
Cholangiocarcinoma			
Ovarian carcinoma			
Pancreatic adenocarcinoma			
Melanoma			
Head and neck squamous cell carcinoma			

*Johnston PA, Grandis JR, Mol Interv. **2011** Feb; 11 (1): 18-26*

Inhibitors of IL-6 receptor complex pSTAT3 Activation: Mechanisms of Action

- **indirectly block the upstream molecules of Stat3 signaling pathway**

- **Antagonists of IL6 binding to IL6Rα**
- **Antagonists of IL6-IL6Rα recruitment of gp130**
- **Inhibitors of IL6 –IL6Rα-gp130 complex activation**
- **JAK Inhibitors**
- **Recruitment of STAT3 in Cytoplasm to pY of activated IL6-IL6Rα-gp130 complex**



- **directly target Stat3 protein, disrupt Stat3 phosphorylation, dimerization, nuclear translocation, and/or DNA binding**

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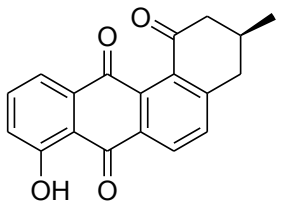
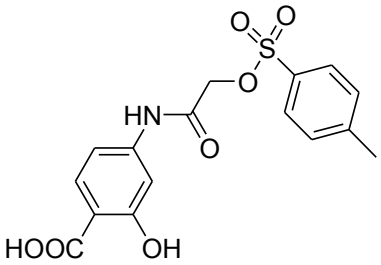
Current Status of STAT3 Inhibitors

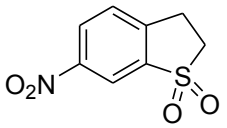
❖ ***Direct target Stat3 protein***



STA-21



S31-201

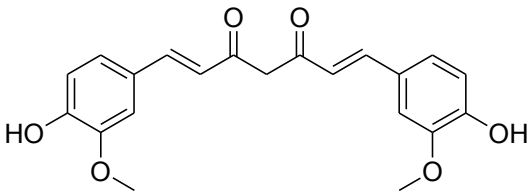


Stattic

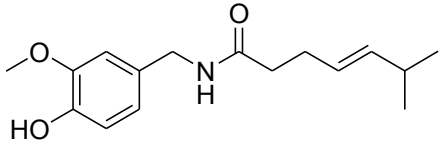
- ***STA-21: inhibits luciferase activity at 20 μM; inhibit STAT3 dimerization, nuclear translocation, STAT3-DNA binding in MDA-MB-435s cells at 20 or 30 μM; inhibit Bcl-X_L and cyclin D1 in MDA-MB-468 breast carcinoma cells.***
- ***S31-201: selectively inhibits STAT3 DNA binding (STAT3:STAT3 IC_{50} = 86 μM, STAT1:STAT3 IC_{50} = 160 μM, STAT1:STAT1 IC_{50} > 300 μM) in EGF-stimulated mouse fibroblasts NIH 3T3/ hEGFR.***
- ***Stattic: inhibits luciferase activity at IC_{50} = 5.1 μM; inhibit STAT3 DNA binding at 10 μM in the nuclear extracts form EGF-stimulated cells; selectively inhibit IL-6 induced pSTAT3 over IFN-γ induced pSTAT1 in HepG2 liver carcinoma cells.***

Current Status of STAT3 Inhibitors

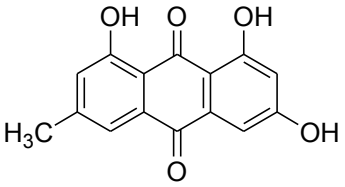
❖ ***indirectly block the upstream molecules of Stat3 signaling pathway***



Curcumin



Capsaicin



Emodin

- ***Curcumin: inhibits JAK2, Src and Erb2, and epidermal growth factor receptor; no selectivity between pSTAT3 and pSTAT1; inhibition of pSTAT3 is reversible.***
- ***Capsaicin: preferentially inhibited constitutive Stat3 phosphorylation in multiple myeloma cells through the inhibition of JAK1 and c-Src.***
- ***Emodin: inhibited Stat3 phosphorylation by targeting JAK2.***

The goal of the STAT3 project is to identify small molecules that selectively inhibit STAT3 over STAT1 signaling and that can be developed into clinical compounds for the treatment of squamous cell carcinoma of the head and neck.

Debnath, B.; Xu, S.; Neamati, N. *J. Med. Chem.* **2012**, *55*, 6645-6668.

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